Science -- First draft

Popovic [redacted]

RESCUE AND CONTINUOUS PRODUCTION OF HUMAN T-CELL LYMPHOTROPIC RETROVIRUS (HTLV-III) FROM PATIENTS WITH AIDS

— way to deal c̄ this
LAV - originally

① Lack of cross hybridiz: c̄ I, II

② " " Ag " reaction

③ Relationship to CIA

④ [illegible] published [illegible]

When the hell are the [illegible]

ABSTRACT

A susceptible and permissive human neoplastic T-cell population is described for routine isolation of cytopathic variants of human T-cell lymphotropic retroviruses (HTLV-III) which can be isolated from pre-AIDS or AIDS patients. The infected T-cell population preserves its capacity for permanent in vitro growth and exhibits continuous virus expression. This system is suitable for isolation of cytopathic variants of HTLV from patients with lymphadenopathy (pre-AIDS) and AIDS, and continuous virus production in high amounts, therefore, enables us to prepare specific viral probes for immunological and nucleic acid studies. The cytopathic effect of HTLV-III can be prepared. One infection is characterized by the induction of multi-nucleated giant cells which can be used as an indicator for the detection of this virus. production.

This abstract is rather trivial for a putative breakthrough paper for Science.

A family of human T-cell lymphotropic retroviruses (HTLV) comprises two major and well characterized subgroups of human retroviruses, called HTLV-I ( ) and HTLV-II ( ). ~~and~~ Recently, a new variant of HTLV ~~has been isolated from~~ a patient with lymphadenopathy named also as lymphadenopathy associated virus (LAV) ( ) which is described here as HTLV-III. The most common isolate obtained from patients with mature T-cell malignancies is HTLV-I ( ). Seroepidemiological and nucleic acid hybridization data indicate that HTLV-I ~~, including its new subtype,~~ is etiologically associated with T-cell leukemia/lymphoma of adults ( ). The disease clusters in the south of Japan ( ), the Caribbean ( ), Africa ( ) and can be found in other parts of the world. HTLV of subgroup II (HTLV-II) was first isolated from a patient with a ~~benign~~ chronic form of a T-cell variant of hairy cell leukemia ( ). To date, this ~~virus represents~~ is the only reported isolate of HTLV-II obtained from a patient with a neoplastic disease. However, isolation of retroviruses and seroepidemiological data suggest that HTLV of both ~~subgroups, including new variants from subgroup III~~, may be ~~involved in the pathogenesis of~~ associated with the acquired immune deficiency syndrome (AIDS) ( ). Here we report development of a system for routine detection & isolation of these and other HTLV variants in patients with AIDS and pre-AIDS

Epidemiologic data strongly suggest that AIDS is caused by an infectious agent which is transmitted by intimate contacts or blood products ( ). ~~To~~ date, over 3000 cases of AIDS have been reported in the U.S. ( ). Patients with the disease include mainly homosexuals ( ), intravenous drug users ( ), Haitian immigrants to the U.S. ( ), and hemophiliacs ( ). Recently, an increased number of AIDS cases have been reported in children whose parents have AIDS or intimate contact(s) with a person having the disease ( ). Although the disease in patients is

Human T-cell leukemia/lymphoma virus

I just don't believe it. We are absolutely incredible

The majority of the isolates belong to a subgroup which is called HTLV-III

and detailed characterization

manifested by opportunistic infections, predominantly Pneumocystis carinii pneumonia and Kaposi's sarcoma, the underlying disorder affects the patient's cell-mediated immunity ( ). ~~The T-cell dysfunction is often marked by an absence of delayed hypersensitivity~~ with absolute lymphopenia and reduced helper T-lymphocyte (OKT4+) subpopulation(s). ~~This results in reverse ratios of helper-to-suppressor T-lymphocyte (OKT4+/OKT8+) counts, poor lymphocyte responsiveness to mitogens ( ). In some cases, a decreased natural killer cell activity was found as well ( ).~~

~~Despite intensive research efforts, the causative agent of AIDS has not yet been identified.~~ Although patients with AIDS are often chronically infected with cytomegalovirus ( ), or hepatitis B virus ( ), we have proposed that the ~~cause~~ of AIDS is a ~~retrovirus~~ from a family of HTLV. ~~This assumption, besides being a well known precedence of causing~~ immune deficiency in cats by feline leukemia viruses ( ), is based on ~~the facts~~ that retroviruses of the HTLV family ~~are characterized by~~ T-cell tropism, ~~and~~ preferentially infect "helper" T-cells (OKT4+), ~~exhibit~~ cytopathic effects on various human and mammalian cells as demonstrated by syncytia induction ( ); ~~and the infection of T-cells by HTLV can lead to an abnormality of a specific~~ T-cell function( ) in some cases may result in a selective cell killing ( ). Moreover, ~~epidemiological~~ studies showed that the presence of antibodies directed to cell membrane antigens of HTLV infected cells is from 30-40% of patients with AIDS ( ). In addition, over 20 HTLV isolates of both subgroups and new variants were obtained from patients with AIDS ( ). The successful detection and isolation of HTLV was made possible by the discovery of TCGF which enables selectively to grow different subsets of normal and

and the development of sensitive assays for retroviral reverse transcriptase based on

neoplastic mature T-cells ( ). The viral rescue and transmission of HTLV into permissive cells followed a well established procedure first worked out in the system of avian sarcoma virus transformed mammalian cells ( ). The cocultivation procedure using cord blood T-cells from newborns as recipient cells for HTLV enabled preferential isolation of HTLV types with immortalizing (transforming) capability ( ). HTLV variants which possess "weak" or lack immortalizing properties for normal T-cells from peripheral blood and exhibit mainly cytopathic effect on them might be more important in the cause of AIDS, in fact such variants were frequently detected but only can only be detected transiently using these normal T-cells as target in cocultivation or cell-free transmission experiments. This was our main obstacle for frequent isolation and particularly for detailed biological, immunological and nucleic acid characterization of these cytopathic variants of HTLV obtained chiefly from patients with AIDS or "pre" AIDS. To overcome these obstacles, we performed an extensive survey for a cell population which would be highly susceptible to and permissive for cytopathic variants of HTLV and would preserve its capacity for permanent growth of the cells after infection with the virus. We report here the establishment and characterization of a new immortalized T-cell population which is susceptible to and permissive for HTLV cytopathic variants and can be used for their routine rescue and continuous production of these retroviruses from patients with AIDS and pre-AIDS.

Several in vitro established permanent cell lines originated from human malignancies were initially assayed for susceptibility to infection with cytopathic HTLV I and II as a reference virus (gift from Dr. L. Montagnier) was used in the first series of experiments. Two cell lines with characteristics of mature T-cells were susceptible to infection with all types of HTLV as determined by reverse transcriptase (RT) assays.

MIKA You are CRAZY

One of them, however, was positive for herpes type virus particles, the second one originated from a patient with mature T-cell malignancy was negative for HTLV infections as well as no viral particles were found by an extensive electron microscopic examination. The [illegible] parental cell line infected by HTLV-III [illegible] virus positive [illegible] for particulate reverse transcriptase activity in the extracellular culture fluids, and about 20% of the infected cell population was positive in indirect immune fluorescent assay (IFA) using serum from a hemophiliac patient (patient E.T.) with lymphadenopathy. The serum of the patient (E.T.) exhibited positivity in [illegible] had antibodies to proteins of disrupted HTLV-III ( ) and also reacted with p61 of HTLV-I and HTLV-II transformed human T-cells in precipitation assays ( ). Since p61 is an envelope precursor of HTLV-I ( ).

In order to obtain a susceptible and highly permissive T-cell population for HTLV-III which in spite of the cytopathic effect of the virus, would preserve permanent growth of these cells and continuous virus production in the presence of the severe cytopathogenic effects of the virus, extensive cloning of the parental T-cell population was performed. A total of 51 single-cell clones were obtained by both capillary ( ) and limited dilution ( ) techniques. The relative growth of these clones were compared after HTLV-III infection.

A representative example of a response to virus infection of 8 T-cell clones which are susceptible to and permissive for one HTLV-III isolate is shown in Table 1. In parallel experiments, 2 X 10^6 cells of each T-cell clone were exposed to 0.1 ml of concentrated virus inoculum containing 10^5 cpm of reverse transcriptase (RT) activity. Then the cell growth, cell morphology, expression of the virus antigen(s) in the cells, and RT activity in culture fluids were assessed after 6 and 14 days of infection. Although all 8 clones were susceptible to and permissive for the virus, as determined by

was [illegible] your problems

One was selected for study after initial studies showed that it was negative for HTLV or for any other viral particles by electron microscopy. When it was determined that [illegible] continuous production of HTLV-III [illegible]

must name the cell line

p.KA has no meaning without conditions + total protein

the fact that a [illegible] patient's sera was reactive with this protein and with proteins of HTLV-III suggest common envelope determinants in HTLV-I, II, & III.

Redundant

IFA for the presence of viral antigen(s) and RT activity in culture fluids, there were considerable differences between infected clones in capability to proliferate after infection. Within 6 days of infection, a cytopathic effect was manifested by a decrease from 10-90% of the initial cell number and, in addition, a high proportion of multinucleated (giant) cells were consistently found in all 8 infected clones. The percentage of T-cells positive for viral antigen(s) determined by immunofluorescent assay in IFA with the patient's serum (from A.I.D.S patient E.T.) and hyperimmune rabbit serum raised against the whole disrupted HTLV-III virus was in the range from 10% to over 80%. After 14 days of infection, total cell number and the proportion of IFA positive cells for the viral antigens increased in all 8 clones. The highest proliferation was found in clone H/4, H/6; and H/3 and lowest was in clone H/3. The virus positive cultures exhibited consistently round giant cells which in Wright-Giemsa staining revealed a high number of nuclei (Fig. 1a). Electron microscopic examinations of the infected cultures showed an abundant number of viral particles (Fig. 1b).

clones with the fastest growth rate also show multinucleated giant cells. These multinucleated giant cells are similar to those induced by HTLV-I and HTLV-II except that the nuclei exhibit a characteristic ring formation that they released considerable amounts of virus

To determine whether HTLV-III is continuously producd by the infected T-cells in long term cultures, both the virus production and cell viability of the HTLV-III infected clone, H4, were followed for several months. As shown in Figure 2a, there was a fluctuation in the amount of virus production, however, culture fluids harvested from the H4/HTLV-III cell cultures at approximately 14 day intervals consistently exhibited particulate RT activity which has been followed for several months. In addition, the viability of the cells was in the range from 65-85% and the doubling time of the H4/HTLV-III, this culture, which is called cell culture was approximately 36-48 hours (data not shown) after 3 weeks of infection. Thus, the data clearly indicate

continuous HTLV-III production by permanently growing T-cell population in long term culture.

Further, the yield of the virus produced by H4/HTLV-III cells was assessed by purification of concentrated culture fluids through a sucrose density gradient and particulate RT activity was determined in each fraction collected from the gradient. As shown in Figure 2b, similar to other retroviruses, the highest RT activity was found at density 1.16g/ml. Electron microscopic (EM) examinations of the aliquots from the fractions with highest RT activity revealed that the banded virus particles at density 1.16g were highly purified. An approximate estimation ( ) of the number of viral particles determined by EM and RT activity suggests that the viral yield from H4/HTLV-III culture fluids is about 10^{11} virus particles per ml. Therefore, the data clearly indicate that the established T-cell clones are susceptible to and highly permissive for cytopathic variants of HTLV; all of them preserve proliferation capacity after infection; and in addition, as demonstrated in the case of H4/HTLV-III clones, most of them can proliferate and continuously produce a large amount of HTLV-III in long term culture.

We have used two clones, H/4 and H/9, for the rescue of cytopathic variants of HTLV from patients with lymphadenopathy (pre-AIDS) or AIDS. As shown in Table 2, these procedures, cocultivation as well as cell-free infection, were effective for virus rescue. HTLV-III isolates have been successfully obtained by cocultivation from (4 patients) and in one case by cell-free infection of T-cell clones (H/4 and H/9) as target cells. In all five cases, the virus release into culture fluids was found by RT assay and extracellular virus particles were detected in 3 cases so far.

More than — additional isolate or detection of HTLV-III have been obtained in our laboratory

all those detected by other techniques will now be adopted to find T-cell clones for [illegible] production & detailed analysis.

[illegible] both sera reacted with acetone-fixed cells, and

and The positivity was between 5 & 80%. the data indicate that

the I-cell clones are suitable for HTLV-III rescue either by cocultivation or by cell-free infection. The transient expression of cytopathic variants

of HTLV in cells from AIDS patients and the necessary lack of a proliferative cell

system which could maintain growth and still be susceptible to and permissive for the virus repre-

sented a major obstacle in detection, isolation, and elucidation of the

precise causative agent of this disease. The establishment of a T-cell population which,

described here, which after virus infection can continuously grow and produce virus, provides

the possibility for detailed biological, immunological and nucleic acid studies of this agent. has opened the way to routinely detect the highly cytopathic variants of HTLV in AIDS and provides the first opportunity for detailed molecular immunological analyses. at

In all cases where this has already been done - the [illegible] infected H/9 and H/9 clones

CONCLUSION NOT COMPLETED

REFERENCES NOT DONE

(per Mika)

Insert - here at end

[redacted]

One [redacted] effect of HTLV-III on cultured multiple [redacted] is the formation of multinucleated in a characteristic ring formation in giant cells of the infected T-cell populations. These structures can be used as an indicator to detect HTLV-III in clinical specimens. This system [redacted] offers the quick, routine detection of HTLV-II and related HTLV cytopathic variants of HTLV

Finally, a ~~[illegible]~~ T- lymphotropic retrovirus different from HTLV-I and II and associated with lymphadenopathy syndrome was detected ~~[illegible]~~ earlier ( ).

We found that this virus, called LAV (provided by ~~[illegible]~~ L. Montagnier & J.C. Chermann) also grows in H4 and produces similar cytopathic effects on it ~~[illegible]~~ as HTLV-III.

~~[illegible]~~ The LAV isolate was reported to be related to equine infectious anemia virus, and ~~[illegible] [illegible] [illegible]~~ the sera from ___ % of patients with AIDS reacted with it. ~~In contrast,~~ ~~HTLV III In [illegible]~~ Every ~~[illegible]~~.

In contrast, HTLV-III is related to HTLV-I + II ~~by nucleic acid [illegible]~~

(... ... ___ et al. This

are reactive with proteins of HTLV-III (

these findings suggest that HTLV-III

and LAV may be different. However, it is

possible that this is due to the insufficient

characterization of

the isolate due to poor virus production.

The question of the relationship of the

various HTLV-III to LAV and to other isolates

from AIDS and pre-AIDS patients

can now be accomplished.

Table 2. Rescue of HTLV-III from Patients with Lymphadenopathy (pre-AIDS) and AIDS

Patient (Initials)	Diagnosis	Origin	Virus Expression			EM
			RT Activity ($\times 10^4$ cpm)	IFA with Rabbit Serum (% Positive)	Human Serum (ET) (% Positive)	
RF*	AIDS (heterosexual)	U.S.	6.3	80	33	+
SN*	Hemophiliac (lymphadenopathy)	Haiti	0.25	10	ND	ND
BK*	AIDS (homosexual)	U.S.	0.24	44	5	+
LS*	AIDS (homosexual)	U.S.	0.13	64	19	+
WT**	Hemophiliac (lymphadenopathy)	U.S.	3.2	69	ND	ND

*Cocultivation with H4 target T-cells

**Cell-free infection

IFA is immunofluorescent assays

EM is electron microscopy.

Their assay indicate that
~~HTLV-I~~ Their ~~isolates~~ are
also T4 lymphotropic
retroviruses with ~~similar~~ many
properties similar to HTLV-I
and I but ~~not cross~~ including
~~reactive with monoclonal~~
antibodies, to ~~HTLV-I or II~~,
~~to purified~~ cross reactivity as determined
with hyperimmune sera to
HTLV-II proteins to HTLV-I ~~and~~
purified p24 but not
with monoclonal antibodies
to HTLV-I p19 or p24-
(See paper 5 ——— et al
this issue). ~~Until a full characterization~~
~~we designate these isolates as all 3 HTLV-III~~
~~although~~ We call all these HTLV-III
it is not yet proven that all are identical.

NOW TURN
BACK OVER.

However, ~~neither~~ HTLV-I and ~~nor~~ HTLV-II have not been routinely isolated ~~found~~ in ~~this disease~~ AIDS or pre-AIDS ~~cases~~. RV serological studies on the other hand suggest that ~~@~~ these patients have antibo